



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:) Examiner: M. WOODWARD
ROGER P. EKINS) Group Art Unit: 1813
Serial No: 07/984,264)
Filed: December 1, 1992)
For: DETERMINATION OF AMBIENT)
CONCENTRATION OF SEVERAL ANALYTES)

#19/dj
9/12/94

DECLARATION OF PROFESSOR ROGER PHILIP EKINS

Commissioner of Patents and Trademarks
Washington, DC 20231

Sir:

I, Professor Roger Philip Ekins, declare as follows:

1. I am the sole inventor of the above patent application (hereinafter the '264 application). At the present time, I am Professor of Molecular Endocrinology at University College and Middlesex School of Medicine in London. My curriculum vitae accompanies this declaration as Exhibit A.
2. I have read and am familiar with the '264 application and the Office Action of 24 March 1994 from the US Patent Office. I wish to comment on some of the technical matters raised in the Office Action and the arguments the Examiner has made therein.
3. An important reference the Examiner relies on in his arguments is my own earlier patent application, WO84/01031, referred to in these proceeding as Ekins '031. The Examiner argues that the present invention is obvious in light of the disclosure in Ekins '031, combined with US 5096807 (Leaback '807). The Examiner argues that Ekins '031 includes an example which uses 0.25V/K moles of binding agent and that it would have been obvious at the priority date of the '264 application to further reduce the

amount of binding agent to the more stringent requirements of this application, namely 0.1V/K moles.

4. I believe this argument does not fairly represent the teaching contained in my earlier application or the prevailing view in the field at the priority date. This is because:

- (a) before the present invention, no-one had realised the importance of using less than 0.1V/K moles of binding agent to assay design;
- (b) there was a clear prejudice amongst those skilled in the art against even trying to reduce the amount of binding agent used in assays;
- (c) there is no suggestion in the above-noted citations that assays using such small amounts of binding agent could be as or more sensitive than conventional assays, attempting to bind most or all of the analyte in a sample.
- (d) there existed a clear prejudice against the use of small amounts of binding agent on the grounds that such use reduced the rate of the reaction between binding agent and analyte, thus prolonging the time required to reach the degree of analyte binding regarded as necessary for the achievement of acceptable sensitivity.

These points are dealt with in more detail below.

5. The Examiner cites my earlier application, Ekins '031, as providing a teaching to the person of ordinary skill in the art to reduce the amount of binding agent to the levels used in the '264 application. However, while Ekins '031 does use smaller amounts of binding agent compared to conventional wisdom in the field (in one example using 0.25V/K moles of binding agent), the aim of this invention was only to make an assay which was sample-volume independent. Certainly, when I made the invention in Ekins

'031, I was not aware of the importance of the use of less than $0.1V/K$ moles of binding agent to assay design and that this provides an assay which removes a small amount of analyte from a sample, regardless of analyte concentration (see figure 1 of the present application).

6. Before the present invention, there was, as far as I am aware, no disclosure or suggestion of an assay - whether of so-called competitive or non-competitive design - using less than $0.1V/K$ moles of binding agent.

On the contrary, it was widely accepted by those expert in the field that maximal sensitivity in "competitive" assays is achieved using binding agent concentrations of $1/K$ or $0.5/K$, and that greater concentrations (binding up to 90% of the analyte) were necessary for highest assay precision, as claimed on theoretical grounds by Berson and Yalow (see Berson, S.A., Yalow, R.S. 1964. *Immunoassay of Protein Hormones*. In: *The Hormones Vol IV* (Eds) G. Pincus, K.V. Thimann, E.B. Astwood 557-630 (Academic Press, New York); Yalow, R.S., Berson, S.A. 1968. *General Principles of Radioimmunoassay*. In: *Radioisotopes in Medicine: in vitro Studies*. (Eds) R.L. Hayes, F.A. Goswitz, B.E.P. Murphy. 7-39 (US Atomic Energy Commission, Oak Ridge, Tennessee); Yalow, R.S., Berson S.A. 1970. *Radioimmunoassays*. In: *Statistics in Endocrinology*. (Eds) J.W. McArthur, T.Colton. 327-344 (MIT Press, Cambridge, MA); Berson, S.A., Yalow, R.S. 1973. *Measurement of Hormones - Radioimmunoassay*. In: *Methods in Investigative and Diagnostic Endocrinology*, 2A. (Eds) S.A. Berson, R.S. Yalow 84-120 (North Holland/Elsevier).

In the case of "non-competitive" assays, amounts of antibody binding the majority of analyte present (ie concentrations in the order of $10/K$ or greater) were regarded as essential for the achievement of highest sensitivity (see, for example, Hay, I.D., Bayer, M.F.

Kaplan, M.M., Klee, G.G., Larsen, P.R., Spencer, C.A. 1991. American Thyroid Association Assessment of Current Free Thyroid Hormone and Thyrotropin Measurements and Guidelines for Future Clinical Assays. Clin. Chem. 37, 2002-2008).

The use of such high binding agent concentrations is widely regarded as the reason for the greater sensitivity and shorter incubation times characterising the non-competitive methods. For these reasons, non-competitive assay methods have largely replaced competitive assay methods in the 1980's. I am therefore certain that, notwithstanding the disclosure in Ekins '031, that assays could be developed that were sample-volume dependent (and that such assays could be useful in certain applications), those of ordinary skill in the art were deeply prejudiced against the use of binding agent concentrations of less than 0.1/K (ie amounts of less than 0.1V/K), or preferably less than 0.01/K, on the grounds that this would lead to a catastrophic loss of sensitivity and yield assays requiring even longer incubation times than required in classical competitive assay methods. I am also certain that, for these reasons, persons of even great skill in the art were not motivated (as the Examiner claims) to reduce the amount of binding agent even below the amounts indicated as permissible in Ekins '031.

7. The Examiner also argues that the person of ordinary skill in the art, when reducing the amount of binding agent in accordance with Ekins '031, could take practical steps to ensure that the sensitivity of the assay was preserved. In effect, the Examiner argues that the person of ordinary skill in the art would perceive that the widely accepted teachings of a Nobel Laureate (Dr Yalow) and of an expert committee of the American Thyroid Association were incorrect, and that assay sensitivity could be retained despite the use of amounts of antibody less by orders of magnitude than those generally regarded as optimal.

8. In effectively asserting that a person of ordinary skill in the art would recognize the teachings of Drs Yalow and Berson and the American Thyroid Association to be obviously wrong, the Examiner also disregards another important consequence of reducing the concentration of binding agent used which would additionally prejudice such a person from using concentrations lower than $0.1/K$ (and preferably less than $0.01/K$). The Laws of Mass Action state that the rate at which an analyte and binding agent react is determined by the product of their respective concentrations, in accordance with the following equation:

$$\text{Rate of formation of bound antigen complex} = K_a[BA][An]$$

where K_a = the association rate constant, and $[BA]$ and $[An]$ are the binding agent and analyte concentrations respectively.

This implies that reduction in the binding agent concentration results in a reduction in the rate of analyte binding, and that smaller amounts of analyte are bound in any given incubation time. The Mass Action Laws also show that the time required to reach equilibrium is increased by a reduction of the binding agent concentration. These considerations have led those skilled in the art to view high binding agent concentrations as "driving" the binding reaction in a forward direction at a high rate, thereby shortening incubation times and the time required to achieve a required sensitivity level. Those concepts underlie the techniques used in modern immunoanalysers, these requiring only a few minutes to perform an assay in contrast to the hours or days necessary in the past using so-called competitive assays relying on binding agent concentrations in the order of $1/K$ - $5/K$.

These considerations would likewise be expected to deter (and did in fact deter) the person of ordinary skill in the

art from using concentrations of binding agent orders or magnitude lower even than those regarded as optimal in a competitive assay, leading him or her to believe that even longer incubation times would be required to achieve acceptable sensitivity.

9. The Examiner also suggests that the reasons why those normally skilled in the art did not in fact exploit certain concepts embodied in Ekins '031, in the way the Examiner retrospectively asserts as being obvious, were that it was not technically possible to construct a sample surface and a device to read the signal, such that amounts of antibody less than 0.1V/K could be utilized. In other words the Examiner seeks to deny the existence of deep-seated prejudice against the use of such small amounts of antibody for the reasons given above, and to claim that a person normally skilled in the art would have used such amounts had the means existed for him or her to do so. This is emphatically not the case. The several alternative techniques used by me to locate microspots of antibody onto plastic surfaces relied on antibody coating methods well known in the art for more than 15 years preceding the date of submission of the above application. Also instrumentation for detecting single or dual fluorescence generated from cells and other small bodies or areas has been in existence for many years. The Examiner's argument on this point is therefore unsustainable, the reality being that sensitive assays based on microspots containing minute amounts of binding agent (less than 0.1V/K moles) were widely regarded as impossible.
10. In summary, my earlier invention in Ekins '031 makes no mention of assay sensitivity, nor does it give any indication that assays of greater sensitivity (and requiring shorter incubation times) than those of classic design could be achieved. It therefore provides no teaching to the person of ordinary skill in the art in the

direction of the present invention, which yields assays of greater sensitivity requiring times of incubation as short as, or shorter than, assays of conventional design, in total contradiction to all accepted ideas in the field.

11. Furthermore, Leaback '807 does not make up for the deficiency in the disclosure of Ekins '031, as Leaback '807 does not contain any disclosure concerning immobilising less than 0.1V/K moles of binding agent as a microspot, nor the advantages that this produces. In particular, Leaback '807 does not disclose or suggest that the use of such an amount of binding agent could provide assays of greater sensitivity, and having incubation times as short as, or shorter than, assays of conventional design using large amounts of binding agent.
12. In summary, the disclosure of Ekins '031 and Leaback '807, considered simply or in combination, make no reference to assay sensitivity and, therefore, provide no teaching in the direction of the present invention to the person of ordinary skill in the art.
13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C 1001 and that wilful, false statements may jeopardize the validity/enforceability of the above noted patent application or any patent issued thereon.

Declared by 

Dated 16 August 1994